Chapter 11
Effect of Low-Frequency Stimulation on Spontaneous Firing in Cultured Neuronal Networks

J. van Pelt, I. Vajda, P.S. Wolters, G. Ramakers and A. van Ooyen

Abstract Cultured neuronal networks from dissociated rat cortical tissue show spontaneous firing activity from about the end of the first week in vitro. Multielectrode recordings have shown slow developmental changes in the firing activity at the individual electrode sites. Here we report that a short period of low-frequency electrical stimulation is able to induce lasting changes in the spontaneous firing activity, significantly larger than developmental changes over similar periods of time.

Introduction

When dissociated rat cortical tissue is brought into culture, neurons readily grow out by forming axonal and dendritic arborizations and synaptic connections. These developing neuronal networks display spontaneous firing activity from about the end of the first week in vitro. Firing rates, recorded with multielectrode arrays, show slow developmental changes, small on time scales of hours [1, 2, 3, 4, 5]. Here we investigated the sensitivity of spontaneous firing activity to short periods of low-frequency electrical stimulation.

Methods

Cell Cultures on Multielectrode Arrays

Dissociated E18 rat neocortical neurons were cultured on planar multi-electrode arrays (MEA) from MultiChannel Systems [2, 6]. Hexa-MEA types were mainly used, with a hexagonal pattern of electrodes with diameters of 10, 20 and 30 μm, respectively. The latter one was used for electrical stimulation.

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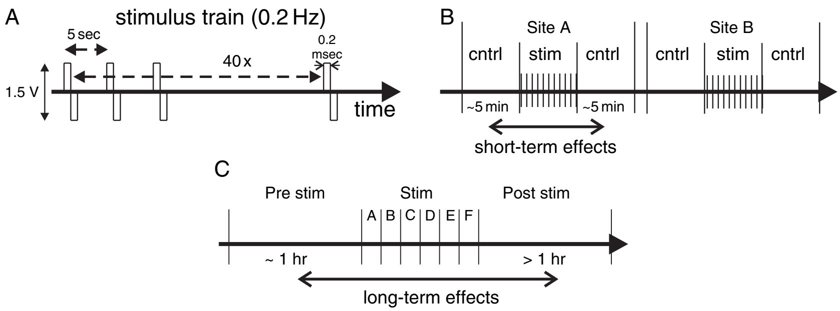

Fig. 11.1 (a) Pattern of low-frequency stimulation, consisting of a train of 40 bipolar pulses of 1.5 V (peak-peak) of 0.2 ms width (single phase), delivered with 5 s intervals (0.2 Hz). (b) Each pulse train is preceded and followed by a period of about 5 min for recording spontaneous activity in the network. (c) Pulse trains are successively applied to six different electrodes in the Hexa MEA multielectrode array

Pattern of Electrical Stimulation

The scheme of electrical stimulation is shown in Fig. 11.1. Individual trains of 40 bipolar pulses at 0.2 Hz were delivered at six successive sites, with a test period of about 5 min before and after each train. Before stimulation at the first site and after stimulation at the sixth site, spontaneous activity was measured for a period of at least 1 h.

Comparing Mean Firing Rates at Individual Sites Between Two Periods

Scatter plots (Fig. 11.2) were constructed in which each individual site is represented by a data point with the mean firing rates for pre-stimulus and post-stimulus periods as coordinates, respectively. Data points at the diagonal line indicate sites with equal firing rates for both periods. The relative deviation *rd* of a data point from the diagonal was calculated as the ratio of its distance to the diagonal and the 3 standard deviation (3σ) distance expected for a Poisson distributed spike train (dashed lines). For a group of data points the mean relative deviation *mrd* was calculated. Data points outside the $\pm 3\sigma$ areas may indicate significant differences in firing rates between the pre- and post-stimulus period.

Results

Long-Term Effects

Figure 11.3 illustrates the long-term effects of low-frequency stimulation on the spontaneous firing rates. The left panel compares the firing rates between the first and the second half of the prestimulus period. All the data points are within the

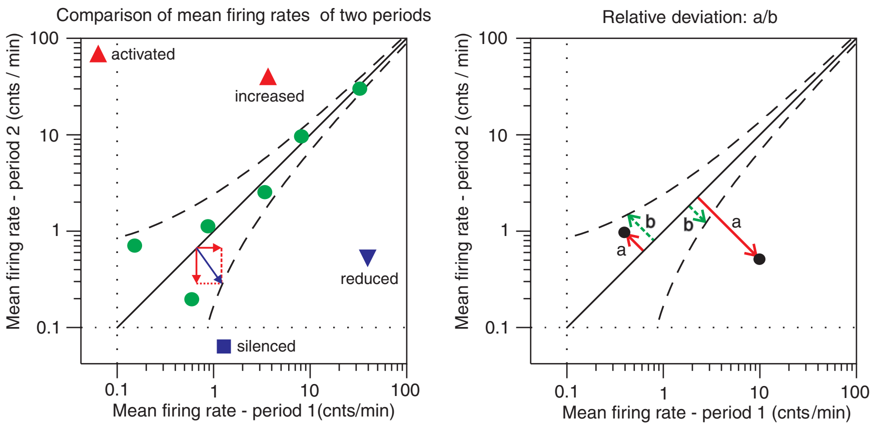

Fig. 11.2 (Left panel) Comparison of mean firing rates at individual sites between pre- and post-stimulus periods. Data points at the diagonal lines indicate sites with equal firing rates for both pre- and post-stimulus periods. The dashed lines indicate 3-standard deviation (SD) boundaries assuming a Poisson distributed spike train. (Right panel) The relative deviation of a data point from the diagonal is expressed by the ratio of the distance of the data point from the diagonal (a) and the 3-SD distance (b)

area bounded by the dashed lines. The central panel compares the firing rates in the second half of the prestimulus period with those in the first half of the poststimulus period. Here, many data points scatter outside the dashed line area, indicating significant changes in firing rates between pre- and post-stimulus period. The right panel compares between the first and the second half of the post-stimulus period. Again all data points are within the dashed lines area indicating that the altered firing rates have maintained in the post-stimulus period. The increased scatter of the data points in the pre-stim post-stim comparison is also shown by the *mrd* values (Fig. 11.4).

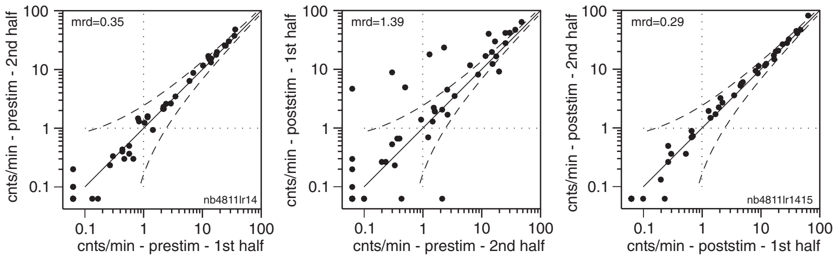

Fig. 11.3 Spontaneous firing rates on individual sites measured one hour before and one hour after a stimulation session. The left panel compares the first half and second half hour of spontaneous activity before the stimulation session. The central panel compares the second half hour of the prestimulation period and the first half hour of the poststimulation period. The right panel compares the first and second half hour of spontaneous activity after the stimulation session. The mean relative deviation (*mrd*) of the data points from the diagonal is indicated in the upper left corner of each panel

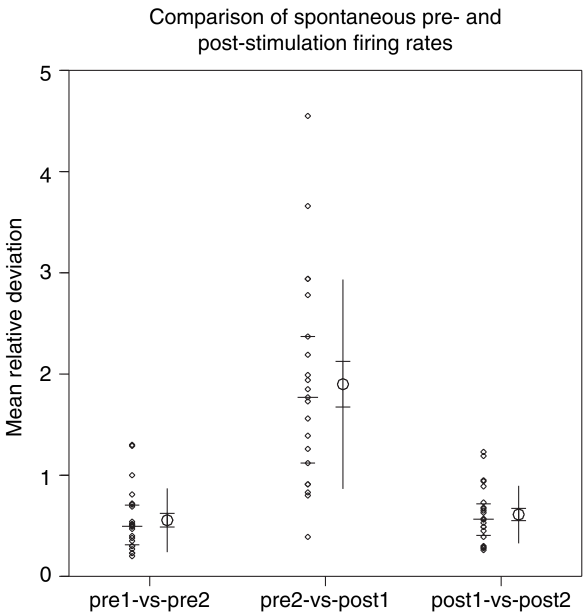

Fig. 11.4 Individual *mrd* values (dots) of 17 stimulus experiments for the pre1-pre2, pre2-post1 and post1-post2 comparisons, as well as their population means (circles) and SD and SEM values (open and closed bars, respectively)

Short-Term Comparison

The results of 12 experiments for the spontaneous firing rates before and after an individual train of 40 stimulus pulses are summarized in the scatter plot of Fig. 11.5. For each experiment, and for each of the six stimulus trains, the spontaneous firing rates in the pre- and post-train periods of 200 sec are plotted as an individual symbol. The symbols appear to scatter equally around the diagonal indicating that there is no systematic difference between pre- and post stimulus firing rates. Several data points scatter outside the dashed lines area.

Discussion

Cultured neuronal networks show ongoing spontaneous activity from the end of the first week in vitro. Firing rates show small developmental changes on time scales of hours [2, 3], indicating a level of robustness in the patterns of spontaneous firing. The present study has shown that low-frequency stimulation is able to induce lasting changes in these firing patterns, significantly larger than developmental changes over the same periods of time (data not shown). Short-term comparisons indicated that even a single stimulus train could be effective. But variable occurrences of network bursts may also have contributed to the scatter in the data points. These findings demonstrate that the intrinsic firing dynamics in cultured neuronal networks maintains a subtle balance between stability under spontaneous conditions and

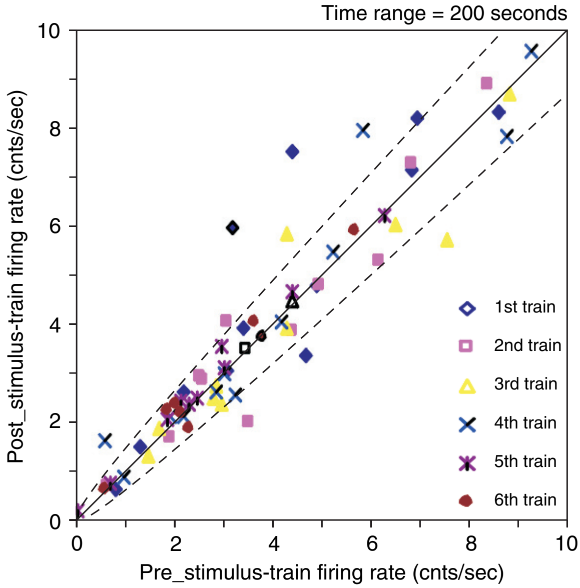

Fig. 11.5 Scatter plot of spontaneous firing rates in a period of 200s before and after a stimulus train. Data from 12 independent experiments. Dashed lines denote 3-SD intervals expected for a Poisson distributed spike train

sensitivity for making transitions to other patterns by even low-frequency external electrical stimulation.

References

1. Shahaf, G. and Marom, S. Learning in networks of cortical neurons. J Neurosci. 21 (2001) 8782–8788
2. Van Pelt, J., Wolters, P.S., Corner, M.A., Rutten, W.L.C. and Ramakers, G.J.A. Long-term characterization of firing dynamics of spontaneous bursts in cultured neural networks. IEEE-TBME 51 (2004a) 2051–2062
3. Van Pelt, J. Corner, M.A., Wolters, P.S., Rutten, W.L.C. and Ramakers, G.J.A. Longterm stability and developmental changes in spontaneous network burst firing patterns in dissociated rat cerebral cortex cell cultures on multi-electrode arrays. Neurosci. Lett. 361 (2004b) 86–89
4. Chiappalone, M., Bove, M., Vato, A., Tedesco, M. and Martinoia, S. Dissociated cortical networks show spontaneously correlated activity patterns during in vitro development. Brain Res. 1093 (2006) 41–53
5. Le Feber, J., Rutten, W.L.C., Stegenga, J., Wolters, P.S., Ramakers, G.J.A. and Van Pelt, J. Conditional firing probabilities in cultured neuronal networks: a stable underlying structure in widely varying spontaneous activity patterns. J. Neural Eng. 4 (2007) 54–67
6. Ramakers, G.J.A., Winter, J., Hoogland, T.M., Lequin, M.B., Van Pelt, J. and Pool, C.W. Depolarization stimulates lamellipodia formation and axonal, but not dendritic branching in cultured rat cerebral cortex neurons. Dev. Brain Res. 108 (1998) 205–216